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"Towards the improvement of rubber tree productivity"

Molecular genetic markers and rubber breeding in Thailand
2 – Field study of the family RRIM600 x PB217 for QTL identification

A. Clément-Demange[1], K. Prapan[2], R. Ratanawong[2], K. Teerawatanasuk[2]

[1]CIRAD Tree crops department –TA 80/01 – Avenue Agropolis, 34398 Montpellier Cedex 5 - France (andre.clement-demange @cirad.fr)

[2]RRIT-DOA, Chachoengsao Rubber Research Center, Chatuchak, Bangkok 10900 - Thailand (rrit@doa.go.th)

Abstract

For developing QTL detection based on the genetic linkage map of the cross RRIM600 x PB217, a clonal field trial was set in Thailand in June 2002. It will be studied until 2010 and targeted to the genetic analysis of the physiological parameters of the latex diagnostic which determine the metabolic typology of rubber clones. This trial, covering an area of 5 hectares, encompasses 196 progenies of the cross as well as the two parents, with every genotype represented by 5 to 16 replicated budded trees. The experimental design is an "alpha-plan" (incomplete block design) : it was chosen for controlling the variation of the environment, for improving the levels of the heritabilities of the measured agricultural traits, and for improving the estimation of the genetic values of every genotype. Any trait with reasonably high heritability can be a candidate for possible QTL identification. So far, tapping has not begun yet, and QTL detection has not been attempted for any trait. In this communication, the characterization of traits related with growth, precocity of wintering, fruiting ability, and tolerance to water stress, is presented as a first characterization of the trial and of the genotypes. As growth is a complex trait, the associated heritabilities appear rather low, with a maximum of 0.36 for one height measurement. As the two parents are roughly equivalent in growth, variation among progenies within this family seems also rather low. This trial provides the rare opportunity to analyse the phenotypic variability displayed within a large size full-sib family of cultivated clones. It will also allow to select the best clones for recommendation to the planters, thereby combining short term and long term methodological and operational applications.

Keywords:
Hevea brasiliensis ; latex production ; metabolic typology of rubber clones ; QTL ; heritability ; growth ; wintering ; fruiting ability ; water stress.

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1. Introduction

Rubber breeding has long been based on conventional breeding, and quantitative genetics based on phenotypic observations. Since recently, molecular genetic markers have brought new possibilities for characterizing genotypes, with view to identify cultivars, to analyse genetic diversity, to establish relationships between agricultural traits and genetic factors (QTLs), and to identify genes of interest.

The general assumption of quantitative genetics (many minor genes determining one trait) is not always true. For the most heritable traits, it is often possible to identify major genes able to determine a significant share of the variation of the traits. QTLs (Quantitative Trait Loci) are specific sites on the DNA genome, tagged by some of the molecular genetic markers that were used in genetic mapping, that indicate the neighbourhood of such genetic factors. QTL identification requires three phases : a) genotyping one segregating family with a large set of markers (genetic mapping), b) phenotyping this family for the targeted traits (field measurements), and c) computerizing genotypic and phenotypic data for analysing the significance of the possible correlations between the genetic markers and the agricultural traits.

Each diploid genotype has two sets of homologous chromosomes. For one marker localized on one locus L, each genotype has two alleles (which can be identical or different). We present hereafter the principle of QTL identification with an example for one marker. The two parents RRIM600 and PB217 may have alleles A and B for RRIM600, and alleles C and D for PB217 at the locus of the marker. Crossing these two clones generates segregation between the alleles through meiosis, and 4 different possible genotypes in the progenies : AC, AD, BC, and BD. Thereby, all the available progenies tested at field level can be distributed among four different groups corresponding to the 4 genotypes. The four groups can be compared for different agricultural traits, and their differences assessed by statistical tests. One significant difference can be assigned to one or some genes in the neighbourhood of the locus that thereby indicates the presence of one QTL, and favourable alleles can be identified for this QTL. This process is performed again for all the markers available on the genetic map, with assistance of a software, and each identified QTL is characterized by its statistical significance and by its contribution share in the explanation of the trait. QTLs provide information about the genetic architecture of the studied agricultural traits (occurrence of some major genetic factors). Theoretically, the genetic markers associated with QTLs can be used for Markers Assisted Selection at a very early stage, at least in the same genetic background. Howeveer, the stability of one QTL over different genetic populations is questionable and must be validated before use in selection.

In order to analyse the genetic bases of latex production, the cross RRIM600 x PB217 was created in Thailand for genotyping the progenies and building a genetic linkage map. The two parents were chosen for their contrasted physiological behaviour, as shown by the metabolic

typology of cultivated rubber clones. A genetic map was built, with 334 progenies, 247 microsatellite markers, and 198 AFLP markers (Prapan et al. 2006).

The main objective of this field trial is to carry out phenotypic measurements with aim to estimate genotypic values as precisely as possible, prior to QTL identification. Following the first successful research for detecting QTLs related with Salb resistance (South American Leaf Blight, Lespinasse et al. 2000), this research is the first attempt in this QTL approach towards analysing the genetic factors of latex production and of tapping and stimulation stress in the Wickham elite breeding population. Moreover, this trial provides the rare opportunity to analyse the phenotypic variability displayed within a large size full-sib family of cultivated clones. It will allow to select the best clones for recommendation to the planters, thereby combining short term and long term methodological and operational applications. This approach represents a good integration of molecular biology and conventional breeding.

2. Materials and methods

The field trial was planted in June 2002 with one-whorl budded plants in bags, and it is 4-year old at the present time. Ages of the trial at the times of the different measurements are hereafter mentioned in months (for instance : m17 = 17-month old). Initially, the weeding was carried out with glyphosate; it appeared that some trees were affected by the chemical and showed modified architecture with 'fox-tailed' shoots (continuous growth instead of rhythmic growth). Glyphosate spraying was stopped after 17-month old. In 2004-2005, a severe drought affected the trees of the trial, causing the death of some trees. In the same period, necroses of the bark and the wood of some trees were observed; they were probably related with the drought.

2.1. Experimental design

It is generally considered that the study of about 200 segregating progenies is reasonably good for QTL detection. The 200 'Codec' codes of the genotypes are from 1 through 196 for 196 progenies, Codec 197 and 198 for RRIM600, Codec 199 and 200 for PB217 (each of the two parents was replicated in two different codes to make sure that these two parents can be observed in the trial). Planting design was 4 x 4 meter (625 trees/ha), a little higher than the usual density of 500 trees/ha, for allowing to plant all the trees required by the experimental design.

The experimental design was chosen for complying with the assessment of a large number of entries (200 Codec codes), with as good as possible control of the variation of the environment. An 'alpha-plan' (incomplete block design, Patterson and Williams 1976) was chosen, with :

- 4 blocks equivalent to 4 complete replications of the 200 entries
- Every block composed of 25 incomplete sub-blocks
- Every sub-block composed of 8 plots equivalent to 8 entries
- Every plot composed of 4 budded trees per plot .

The total number of sub-blocks is 4 x 25 = 100.
The total number of plots is 4 x 25 x 8 = 800.
The initial total number of budded trees is 4 x 25 x 8 x 4 = 3200.

The distribution of the 200 entries among the 100 sub-blocks was managed by a Cirad software (jean-pierre.jacquemoud-collet@cirad.fr) in order to comply with the following condition : Any entry 'A' is set once in each of 4 different sub-blocks; 'A' can be directly compared to 7 other entries in every sub-block, and 'A' can be directly compared to 7 x 4 = 28 different entries among the 4 sub-blocks where 'A' is set ('A' is the only common entry to the 4 sub-blocks). This condition is theoretically important for calculating adjusted means of the different entries, and thereby optimising the estimation of genotypic values.

2.2. Field observations

Field observations of the architecture of the genotypes were made regularly for a qualitative description of the branching habit of the different genotypes. But a rigorous characterization of the architecture, that would have been based on Cirad 'Amap-mod' measurement procedures, could not be carried out due to the lack in manpower. Numeric photos of the crown of each tree were taken systematically in months 23 and 31 for keeping a memory of the initial aspects of the trees.

2.3. Field measurements

During the first four years of the trial, the following variables were measured:

- Diameter of the trunk at 10-cm high: in months 6, 12, 18, and 23 (V7, V9, V16, and V19 on 2852, 2991, 2508, and 2508 trees respectively)
- Girth of the trunk at 1-meter high: in months 18, 23, 31, 36, 43, and 47 (V17, V20, V26, V38, V47, and V50 measured on 2508, 2508, 2504, 2296, 2427, and 2427 trees respectively)
- Girth of the trunk at 1.7-meter high: in months 18, 23, 31, 36, 43, and 47 (V18, V21, V27, V39, V48, and V51 measured on 2508, 2508, 2504, 2296, 2427 and 2427 trees respectively)
- Height of the trees: in months 6, 12, 17, 23, 30, 36, 43, and 47 (V8, V10, V14, V22, V24, V37, V46, and V49 measured on 2852, 2991, 2520, 2508, 2504, 2296, 2427 and 2427 trees respectively).

A global analysis of the evolution of the growth in height and girth over the first four years was carried out on 1950 trees.

At 12-month old, the ratio 'height/diameter' (V10/V9) was calculated for 2502 trees (variable V13).

The height increment from 12 to 17-month old (V14 – V10) was calculated on 2520 trees (variable V14V10).

At 31-month old, the height of the insertion on the trunk of the lowest ramification was measured on 2503 trees (variable V29a). The difference between the total height (V24) and the lowest ramification (ramified height, V29b) was calculated. The ratio 'ramified height / total height' was calculated (V29b/V24 = V29c).

At 36-month old, the largest width of the crown was measured on 2296 trees (variable V40).

At 42-month old, the number of living branches inserted on the trunk below 4-meter high was counted (variable V45) for 1959 trees with trunk higher than 420 cm.

At 12, 24, and 36-month old (1, 2, and 3-year old), samples of 25 trees belonging to the family RRIM600 x PB217 were successively extracted from the plot just neighbouring the trial for assessing the variation of the different components of biomass of the trees and the evolution of biomass production along time. Multiple linear regressions were set for estimating the total dry weight of the aerial part of all the trees in the trial

Scoring methods were used for assessing: a) wintering earliness (defoliation-refoliation) in month 31 (January 2005), b) abundance of fructification in month 36 (June 2005), and c) susceptibility to water-stress as observed after a severe drought in month 36 (June 2005). The scoring methods were as following:

a) Wintering earliness (defoliation-refoliation): '1' = not yet defoliated; '2' = defoliating; '3' = defoliated; '4' = refoliating; '5' = completely refoliated. Means were calculated for the 200 entries; they can vary from 5.0 (very early wintering) to 1.0 (very late wintering). This observation, performed at only one date in January 2005, allows to rank the genotypes. But the dates of the beginning and of the end of the wintering process were not recorded.
b) Fruiting ability: '5' = many fruits; '3' = at least one fruit; '0' = no fruit. An index V33 was calculated for estimating the ability to produce fruits for each genotype: the number n1 of trees scored '3' were counted for half; the number n2 of fruits scored '5' were counted for full; the total number of trees per genotype is N; V33 = (n1+n2)/N. V33 varies from 0.00 (no fruit) to 0.75 (many fruits) over 2352 trees. Means were calculated for the 200 entries.

c) Susceptibility to water stress: ‘5’ = healthy canopy; ‘4’ = yellowish leaves, or small leaves exhibiting signs of stress, or with some dry shoots; ‘3’ = some branches without leaves; ‘2’ = a large part of the canopy without leaves, top dead, but at least one branch with leaves; ‘1’ = dead tree excepted for some shootings at the base of the tree. An index V32 was calculated for each genotype: trees scored ‘1’ are affected with the coefficient 1, trees scored ‘2’ with 0.75, trees scored ‘3’ with 0.50, trees scored ‘4’ with 0.20, and trees scored ‘5’ with 0.00 (total divided by the number of trees per genotype). A32 varies between 0.000 (tolerant genotype) and 0.500 (susceptible genotype) over 2508 trees. Means were calculated for the 200 entries.

2.4. Statistical analyses

Statistical analysis was carried out with ‘Sas’ software. ‘Glm’ procedure was used for variance analysis, calculation of the % coefficient of variation of the error (CVe%), and calculation of the coefficient of determination R^2 (% of the total variation explained by the statistical model). ‘Lsmeans’ procedure was used for calculating the adjusted means of the genotypes which represent the estimations of the genetic values for each variable. ‘Varcomp’ procedure was used for estimating the variance components and deducting heritabilities of the different variables.

The general linear model associated to the experimental design is composed of 4 independent sources of variation that are estimated by the ‘Varcomp’ procedure of ‘Sas’ software:

- Codec (G): variation between the genotypes (200 entries)
- Rep: variation between the 4 blocks
- Block(rep): variation between the 25 sub-blocks within every block
- Error (E): uncontrolled variations and interactions between the 3 controlled sources.

The large sense heritability h^2l is the ratio G / (G+E).

Because many measurements cannot be carried out on a large number of trees, the available measured variables were used for assessing the reduction in heritability with reduced samples of trees. A first estimation was done with all the available trees (about 2500 trees). A second estimation was done with a sample of the two tallest trees in every plot (sometimes, there is only one tree left in the plot); this sample comprised 1469 trees. A third estimation was done with a sample of the tallest tree in every plot; this sample comprised 785 trees.

Statistical analyses were carried out only on measured variables (not on scoring).

3. Results

3.1. Variations in the growth traits and heritabilities

For the different growth traits that were measured, table 1 shows the coefficients of variation of the error (CVe%) as estimated by the variance analysis, the coefficients of determination of the statistical models, the different components of the total variance, and the estimated heritabilities.

The coefficient of variation of the error varied from 39 % to 8 %, depending on the variables. For a type of variable (i.e. diameter, or girth, or height, etc.), there was a reducing trend along time. As an example, for height, CVe% = 24 % at 6-month old, and 9 % at 47-month old. In relation with this, the share of variation explained by the statistical model, R^2, tended to increase along time (from 0.50 to 0.64 for height).

The variance component due to the 4 replications varied between 0 % and 22 %. The variance component due to the sub-blocks nested within the four replications varied between 1 % and 13 %. The two components provided a small contribution to the variation. By contrast, the uncontrolled variance due to the error varied between 52 % and 90 %. The variance due to the genetic differences between the 200 codes varies from 0 % to 30 %. In most cases, the genetic difference between the 200 codes was very highly significant. There was no significant difference between the genotypes only for the measure of the largest length of the crown of the trees.

Large sense heritabilities varied from 0.00 to 0.36 which seems rather low for QTL identification. As a matter of fact, growth as measured here is a very complex trait, associating and cumulating many different factors along time.

In every case, the confidence intervals of the adjusted means of the measured traits were rather large. As a example, the general mean of the height at 47-month old (variable V49), was of 597 cm. For any genotype, the standard deviation of the adjusted mean was about 35 cm, and the standard error was 35 x 1.96 = 69 cm ($\alpha = 0.05$); thereby there was a probability of 95 % that the real genetic value was comprised between 528 and 666 cm.

Table 2 shows three estimations of heritabilities for different variables, depending on the number of measured trees. Even if the best trees were chosen within the trial, the reduction of the number of measured trees clearly reduced the level of heritability due to a lower control of the variation of the error.

3.2. Height, girth, and biomass measured on extracted trees

Table 3 provides the most important data issued from measurements after extraction of 25 trees every year at one-year old, two-year old, and three-year old.

At one-year old, the trees were still very small with fresh weights varying from 100 to 1300 grammes. The attempt to predict the dry weight of the trunk at 12-month old, on the basis of a multiple linear regression, taking into account trunk diameter and height, showed a high determination coefficient ($R^2 = 0.98$), but a high level of variation of the error (CVe% = 39 %), and a low heritability ($h^2l = 0.11$).

At two and three-year old, the trees were comprised in the same range of height (from 4 to 6-meter high), and the fresh weights varied from 4 to 35 kgs depending on height and girth. The 50 trees extracted in 2004 and 2005 were used for designing a linear regression and predicting the fresh weight of the total biomass of the trees (including roots).

Total fresh weight = 12943 + 31.65 height -2.62 vol + 4227 S1 – 4644 G1 ($R^2 = 0.93$)

With:

- Height = variable V37 measured at 36-month old
- G1 = variable V38 measured at 36-month old (girth measured at 1-meter high)
- S1 = basal area at 1-meter high, deducted from V38 = girth G1 measured at 1-meter high (m36)
- Vol = S1 x height

The prediction was performed on the measurements of 2121 trees at 3-year old, with $R^2 = 0.93$. The heritability was $h^2l = 0.21$, equivalent to the heritability found for the girth measurement at 1-meter high.

Figure 1 shows the contributions of the different components to the formation of the total fresh weight of the trees extracted in June 2005, at three-year old. In average, the shares of the different components were as following:

- taproot: 19 % (from 13 % to 31 % depending on the trees)
- lateral roots: 6 % (from 3 % to 9 %)
- trunk: 37 % (from 20 % to 54 %)
- branches of second order: 13 % (from 5 % to 22 %)
- branches of upper order: 8 % (from 0 % to 21 %)
- leaves: 14 % (from 5 % to 22 %)
- fruits: 3 %. (from 0 % to 21 %)

3.3. Diameter of the trunk at 10-cm high

The evolution of the trunk diameter measured at 10-cm high (figure 2) showed three periods: a) dry period from 6 to 12-month old, b) rainy period from 12 to 18-month old, and c) dry period from 18 to 23-month old. The level of the two parents was intermediate between the extreme growths (minimum and C33 on the one hand, and maximum and C56 on the other

hand). At 23-month old (nearly 2-year old), the variation in diameter was comprised between 3.1 and 5.3 cm.

3.4. Girth of the trunk, at 1.0-meter high, and at 1.7-meter high

The increase in the girth of the trunk, as measured at 1-meter high and 1.7-meter high (figure 3 - A and B) varied depending of the dry and the rainy seasons. The average girth increment (measured at 1-meter high between 18 and 47-month old) varied between 3.6 and 5.4 mm per month for the extreme genotypes. At 47-month old, the adjusted means of the genotypes for the girth measured at 1-meter high varied between 20.4 and 28.6 cm; the coefficient of variation between these adjusted means was of 7 %.

3.5. Total height

The evolution of the total height of the trees (figure 4-A) clearly showed 2 phases: a) a first phase of fast growth until 17-month old, and b) a second slower growth phase from 17 to 47-month old. The slower growth from 17 to 47-month old can be explained by three reasons:

- the roots met a hard pan located at 50 cm below ground level
- there was a severe drought during the period from 23 to 36-month old
- from 36 to 47-month old, the canopies of the trees begin to meet each other, and competitions become important.

The two codes used for each of the two parents exhibited rather different growths, which indicate that the number of four plots per genotype probably is rather small for a precise characterization of each genotype. At 47-month old, the height varied between 518 and 743 cm. The variation coefficient of the adjusted mean heights, between the adjusted means of the genotypes at 47-month old, was of 7 %.

Figure 4-B shows that the height increment between 17 and 47-month old was negatively correlated with the height at 17-month old ($r = -0.37$, $df = 198$, highly significant). However, only $R^2 = 14$ % of the variation in the height increment was predicted by the height at 17-month old.

3.6. Number of branches below 4-meter high

At 42-month old, the variation in the number of branches inserted on the trunk below 4-meter high (N4) for trunks higher than 420 cm, was of 0 to 39 branches. A highly significant negative correlation was shown between mean height and mean number of branches over the 27 considered classes (figure 5-A, $r = -0.90$, $df = 25$). This negative correlation was also shown over the 200 genotype-codes (figure 5-B, $r = -0.18$, $df = 198$), with means calculated over about 10 trees per genotype. Heritability of N4 was estimated as $h^2l = 0.24$.

3.7. Wintering earliness

The variation in the wintering earliness was characterized for the 200 genotype codes at 31-month old (figure 6). The two parents PB217 and RRIM600 appeared to be medium, with PB217 a little earlier than RRIM600. There was no relationship between wintering earliness and growth.

3.8. Fruiting ability

Fruits were noted for the first time at 24-month old, on 16 out of 2519 trees observed in this trial, and on 11 different genotypes (figure 7). At 36-month old, fruits were noted on 666 trees out of 2352 observed trees. The index of fruiting ability, issued from these observations, showed a wide genetic variation in this trait. RRIM600 appeared as medium. Considering PB217, one replication was medium, while the other showed poor fruiting ability. There was no relationship between fruiting ability and growth.

3.9. Susceptibility to water stress

The severe drought during the third year (2004-2005), combined with the hard pan below ground level caused an important loss of trees in the trial. Over a total number of 2508 trees, 176 trees died (7 %), 143 were still living but very severely affected on a large part of the trees (6 %), 91 trees were significantly affected, and 82 trees showed light symptoms. Overall, 20 % of the trees showed visual symptoms of the water stress. Figure 8 shows the variation between the genotypes, as expressed by an index of the stress.

The observation of severe necroses affecting the bark and the wood at the bottom of the trunk of about 50 trees in the trial was probably related with this drought. Some of these trees were then broken at the level of the necrosis.

4. Discussion

The ecological site of Chachoengsao is rather hard for rubber growth, as compared with the most favourable rubber cropping areas. The annual rainfall, with 1315 mm in 2002, 1287 mm in 2003, and only 1062 mm in 2004, appears to be limiting as compared to evapo-transpiration, especially during the 4 to 5 months from December to April (dry season and wintering). In this trial, water stress was emphasized by the hard pan lying 50 cm below ground level. Girth measured at one-meter high at 47-month old was about 24 cm for the two parents RRIM600 and PB217. As a comparison, the equivalent girths in southern Côte d'Ivoire, a favourable site for rubber growth, were around 34 cm for RRIM600 and PB217, with an age of opening between 5.5 and 6-year old. With an average girth increment of 5 cm per year, the normal opening of RRIM600 and PB217 in the conditions of this trial in the site

of Chachoengsao would be between 7 and 8-year old (with opening at a girth of 50 cm, measured at 1-meter high).

All the variables submitted to statistical analysis are describing growth which is a very complex trait. As a consequence, heritabilities are rather low. The maximum h^2l (0.36) was obtained for height measured at 30-month old. These moderate heritabilities would probably be not high enough for a successful QTL detection. Moreover, the variance between the progenies, expressed by the variation coefficient between the adjusted means of the genotypes (6 % to 8 % for the height and girth measurements at 47-month old), seem rather low, which can be related with the fact that the progenies belong to the same full-sib family in the domesticated Wickham population, and also to the fact that the two parents have roughly the same growth fastness. Segregation of the genetic factors between the two parents in recombination does not appear very high for these growth traits.

Prediction of biomass, by destructive measurements and based on height and girth measurements, provided a poor heritability at one-year old with only 25 extracted trees. But biomass predicted by the joint use of 50 trees extracted at two and three-year old provided an heritability of the same level as girth measurements; this prediction could be increased by the introduction of another variable related with the abundance of branching. Traits that were scored such as wintering earliness, fruiting ability, and susceptibility to water stress could not be analysed in another way than ranking the genotypes according to mean values; nevertheless, these traits can be also submitted to QTL detection.

As RRIM600 and PB217 are physiologically very different, it can be hoped that segregation will be more important for the latex production and the latex diagnostic parameters, as measured after the beginning of tapping.

As the main objective of this trial is QTL detection, there is a compromise between the total number of observed genotypes, the number of budded trees for each genotype, and the time necessary for measuring the different traits. For traits that are difficult to measure, the number of trees can be reduced to a minimum of about 800 trees (one tree per plot), but it was shown that this reduction is paid by a reduction in heritability, thereby generating the risk of missing some possible QTLs.

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Table 1 - CVe%, R^2, variance components and large sense heritabilities of the different measured growth traits up to 47-month old ('Sas', 'Glm' and 'Varcomp' procedures).

Table 2 - Three estimations of heritabilities, depending of the number of measured trees.

Table 3 – Data from extracted trees at one, two, and three-year old. Height (cm), girth at 10-cm high (cm), girth at 1-meter high (cm), and fresh weight of the trees (grammes).

Figure 1 - The contribution of the different components (taproot, lateral roots, trunk, branches of second order, branches of upper orders, leaves, and fruits) to the formation of the total fresh weight of the 25 trees extracted in June 2005 (36-month old).

Figure 2 - Evolution of the trunk diameter measured at 10-cm high from 6 to 23-month old (cm).

Figure 3 - Evolution of the trunk girth measured: A) at 1-meter high, and B) at 1.7-meter high from 18 to 36-month old (cm).

Figure 4 - Evolution of the total height of the trees from 6 to 36-month old (cm).

Figure 5 - A) Relationship between the total height and the number of branches below 4-meter high at 42-month old (N4 counted over 1959 trees and distributed among 27 height-classes). B) Relationship between total height and N4 among the 200 genotype-codes.

Figure 6 - Wintering earliness as observed at 31-month old during the dry season (January 2005). A) Variation of the index by decreasing rank over the 200 genotypes. B) Wintering earliness of some genotypes, including the parents RRIM600 and PB217.

Figure 7 - Variation of fruiting ability at 3-year old. A) Variation of the index by decreasing rank over the 200 genotypes. B) Fruiting ability of some genotypes, including the parents RRIM600 and PB217.

Figure 8 - Variation of the susceptibility to water stress as observed after the severe drought of 2004-2005. A) Variation of the index by decreasing rank over the 200 genotypes. B) Susceptibility to water stress of some genotypes, including the parents RRIM600 and PB217.

Table 1: CVe%, R^2, variance compoments and large sense heritabilities of the different measured growth traits up to 47-month old ('Sas', 'Glm' and 'Varcomp' procedures).

Code-var	Definition	Nb of trees	CVe%	R^2	Codec (G) %	Rep %	Block(rep) %	Error (E) %	h^2l
V7	Diameter at 10 cm high, m6	2852	18	0.49	10	1	7	83	0.11
V9	Diameter at 10 cm high, m12	2991	16	0.50	10	3	6	81	0.11
V16	Diameter at 10 cm high, m18	2508	10	0.61	30	7	3	60	**0.33**
V19	Diameter at 10 cm high, m23	2508	10	0.61	26	6	7	61	**0.30**
V17	Girth at 1.0 m high, m18	1950	11	0.58	24	4	4	68	**0.26**
V20	Girth at 1.0 m high, m23	1950	11	0.56	19	4	5	72	**0.20**
V26	Girth at 1.0 m high, m31	1950	9	0.60	20	11	5	64	**0.24**
V38	Girth at 1.0 m high, m36	1950	9	0.58	19	5	6	69	**0.22**
V47	Girth at 1.0 m high, m43	1950	9	0.60	16	15	6	64	**0.20**
V50	Girth at 1.0 m high, m47	1950	8	0.62	18	17	5	60	**0.23**
V18	Girth at 1.7 m high, m18	1950	13	0.56	22	4	2	73	**0.23**
V21	Girth at 1.7 m high, m23	1950	13	0.55	18	5	3	74	**0.20**
V27	Girth at 1.7 m high, m31	1950	9	0.60	18	14	4	64	**0.22**
V39	Girth at 1.7 m high, m36	1950	9	0.60	16	11	10	64	**0.20**
V48	Girth at 1.7 m high, m43	1950	10	0.62	13	19	7	60	0.18
V51	Girth at 1.7 m high, m47	1950	9	0.61	14	20	5	61	0.19
M18	Mean of girths measured at 1 and 1.7-meter high, m18	1950	12	0.57	23	4	3	70	**0.25**
M23	Mean of girths measured at 1 and 1.7-meter high, m23	1950	11	0.56	20	5	4	71	**0.22**
M31	Mean of girths measured at 1 and 1.7-meter high, m31	1950	9	0.61	19	13	4	64	**0.23**
M36	Mean of girths measured at 1 and 1.7-meter high, m36	1950	9	0.60	18	8	8	65	**0.22**
M43	Mean of girths measured at 1 and 1.7-meter high, m43	1950	9	0.61	15	18	6	61	0.19
M47	Mean of girths measured at 1 and 1.7-meter high, m47	1950	9	0.62	16	19	5	60	**0.21**
V8	Height, m6	1950	24	0.50	13	0	4	82	0.14
V10	Height, m12	1950	20	0.45	7	1	2	90	0.07
V14	Height, m17	1950	12	0.58	19	3	8	70	**0.21**
V22	Height, m23	1950	11	0.61	23	4	8	65	**0.26**
V24	Height, m30	1950	8	0.68	29	7	11	52	**0.36**
V37	Height, m36	1950	8	0.66	29	3	12	56	**0.34**
V46	Height, m43	1950	8	0.64	21	15	7	57	**0.27**
V49	Height, m49	1950	9	0.64	18	22	4	56	**0.24**

Table 1 : Following …

Code-var	Definition	Nb of trees	CVe%	R^2	Codec (G) %	Rep %	Block(rep) %	Error (E) %	h^2l
V11	Square-Root(Dwt), dry weight of the trunk (calculated), m12	2989	39	0.50	10	4	6	80	0.11
V13	Height/Diameter ratio V10/V9, m12	2502	11	0.50	10	1	1	87	0.10
V14V10	Height increment from m12 to m17	2520	19	0.55	15	4	4	77	0.16
V29a	Height of lowest ramification, m31	2503	11	0.51	14	6	3	78	0.15
V29b	Difference between total height and lowest ramification, m31	2503	18	0.55	21	4	8	67	**0.24**
V29c	Ratio of ramified height / total height, m31	2503	13	0.44	8	0	4	88	0.08
V40	Largest width of the crown, m36	2296	16	0.56	0	21	13	66	0.00
V45	Number of branches below 4 meter high, m42	1959	34	0.57	24	2	4	71	**0.25**

Table 2: Three estimations of heritabilities, depending of the number of measured trees.

Code-var	Definition	S1 (785 trees)	S2 (1469 trees)	Max number of trees	Nb of trees
V24	Height, m30	0.30	0.37	0.37	2504
V16	Diameter at 10 cm high, m18	0.24	0.30	0.33	2508
V37	Height, m36	0.28	0.31	0.33	2296
V19	Diameter at 10 cm high, m23	0.23	0.27	0.30	2508
V22	Height, m23	0.19	0.25	0.27	2508
V17	Diameter at 1.0 m high, m18	0.15	0.22	0.26	2508
V20	Diameter at 1.0 m high, m23	0.18	0.23	0.25	2508
V26	Girth at 1 m high, m31	0.18	0.21	0.25	2504
V27	Girth at 1.7 m high, m31	0.17	0.23	0.24	2504
V21	Diameter at 1.7 m high, m23	0.15	0.21	0.24	2508
V29b	Difference between total height and lowest ramification, m31	0.14	0.21	0.24	2503
V18	Diameter at 1.7 m high, m18	0.13	0.21	0.23	2508
V39	Girth measured at 1.7 m high, m36	0.11	0.18	0.22	2296
V38	Girth at 1 m high, m36	0.13	0.19	0.22	2296
V14	Height, m17	0.18	0.25	0.18	2520
V8	Height, m6	0.07	0.13	0.15	2852
V29a	Height of lowest ramification, m31	0.08	0.12	0.15	2503
V11	Square-Root(Dwt), dry weight of the trunk (calculated), m12	0.04	0.09	0.11	2989
V9	Diameter at 10 cm high, m12	0.02	0.10	0.11	2991
V7	Diameter at 10 cm high, m6	0.04	0.11	0.11	2852
V10	Height, m12	0.01	0.07	0.10	2991
V13	Height/Diameter ratio V10/V9, m12	0.04	0.13	0.10	2502

Table 3 – Data from extracted trees after one, two, and three years. Height (cm), girth at 10-cm high (cm), girth at 1-meter high (cm), and fresh weight of the trees (grammes). Fresh weight of trees without roots at one-year old, total fresh weight at two and three-year old.

One-year old - June 2003					Two-year old - June 2004						Three-year old - June 2005				
Tree code	Height cm	G-0.10m cm	Fwtree g		Tree code	Height cm	G-0.10m cm	G-1.00m cm	Fwtree g		Tree code	Height cm	C-0.10m cm	C-1.00m cm	Fwtree G
11	340	9.3	1238		24	614	22.0	16.5	11800		9	603	26.8	21.8	34745
6	290	8.0	1039		22	601	18.0	16.0	11500		10	594	30.5	22.8	33155
19	320	8.3	928		21	491	20.5	16.0	11300		5	521	27.9	20.3	28958
9	310	8.6	905		14	595	17.5	15.0	9470		7	510	26.9	20.0	26780
4	310	8.6	904		19	620	17.0	15.0	9220		6	421	22.7	17.2	23225
10	295	8.3	863		10	428	17.0	14.0	9100		14	619	23.5	18.6	22020
13	300	7.9	853		11	495	18.0	13.5	9060		1	549	24.0	18.8	21845
3	280	8.8	840		25	555	17.0	15.5	8910		19	577	22.3	17.8	21680
18	270	8.9	821		17	480	18.0	14.5	8490		13	533	22.5	18.0	20780
5	265	7.8	818		8	511	15.5	13.8	8480		8	570	22.9	18.5	19380
16	225	7.4	628		6	550	14.5	13.2	8470		20	545	21.2	17.5	19380
12	270	7.7	596		16	443	17.0	14.5	8300		4	590	22.1	19.0	18838
8	240	7.5	578		23	538	16.5	14.0	8060		23	552	20.9	16.5	15405
7	265	7.0	576		18	539	16.5	14.0	7840		15	651	24.6	20.1	15135
24	215	7.1	540		13	352	16.0	14.0	7800		2	529	20.9	16.4	14690
25	210	6.2	508		20	533	17.5	14.0	7800		11	647	20.3	16.7	14530
22	220	7.4	496		7	395	15.0	12.5	7510		25	484	19.3	15.0	12640
17	200	7.5	437		9	398	17.0	13.0	6850		12	478	19.5	15.4	12130
20	200	6.1	421		15	478	17.0	13.8	6580		17	412	18.9	14.6	11675
23	200	6.2	412		3	312	14.6	11.7	6100		21	446	18.4	14.5	11665
2	160	4.1	296		1	322	13.0	10.0	6030		24	491	16.8	13.5	10986
14	165	5.2	281		2	542	13.1	12.0	5940		22	484	18.4	14.3	9510
21	130	4.4	241		5	472	14.7	10.8	5850		16	380	15.5	12.7	7585
15	140	4.8	212		12	454	15.0	11.0	5330		18	381	14.4	11.0	6945
1	70	2.9	100		4	411	13.5	11.9	4700		3	402	12.5	8.5	4210
Mean	**236**	**7.0**	**621**			**485**	**16.5**	**13.6**	**8020**			**519**	**21.3**	**16.8**	**17516**

Figure 1 – The contribution of the different components (taproot, lateral roots, trunk, branches of second order, branches of upper orders, leaves, and fruits) to the formation of the total fresh weight of the 25 trees extracted in June 2005 (36-month old).

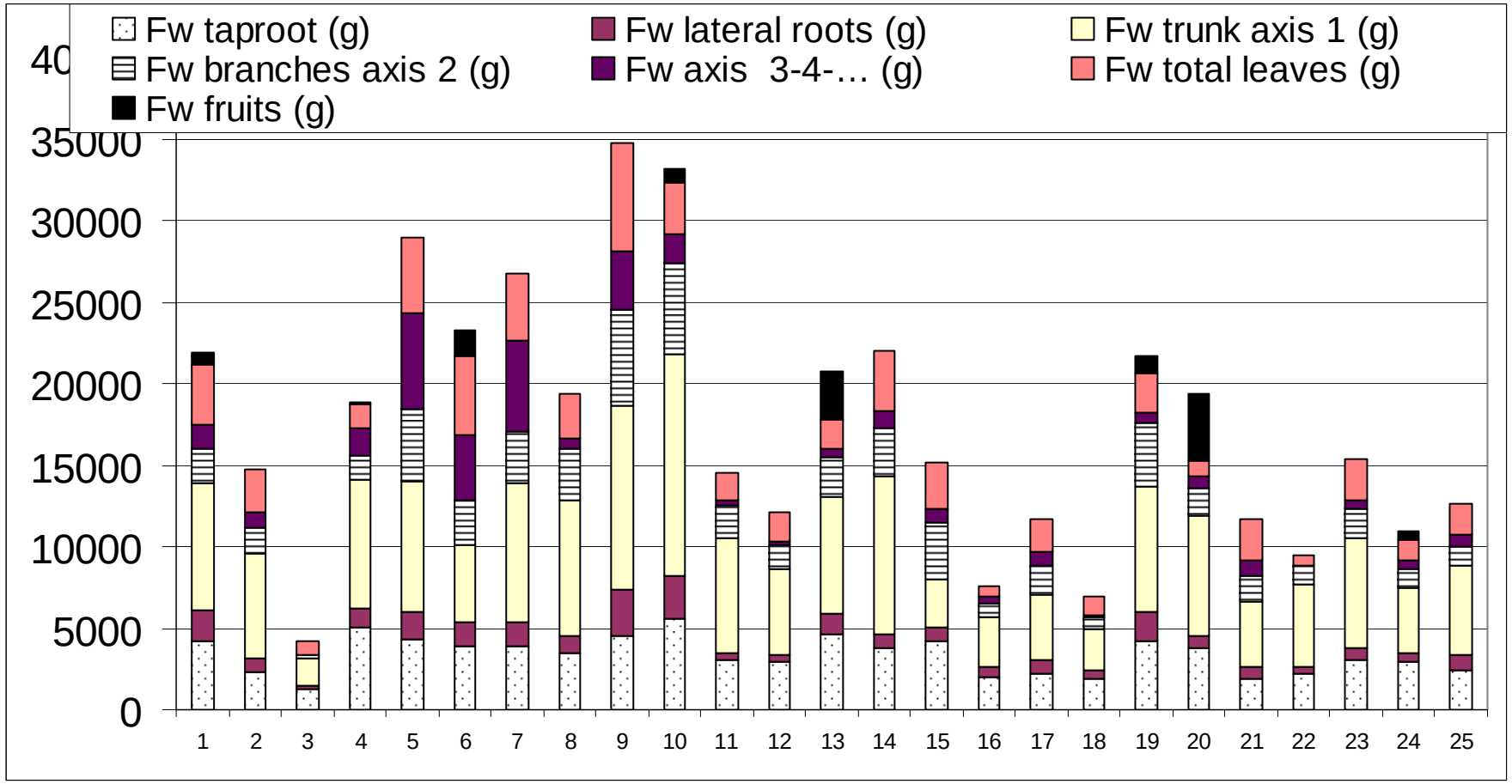


Figure 2 - Evolution of the trunk diameter measured at 10-cm high from 6 to 23-month old (cm).

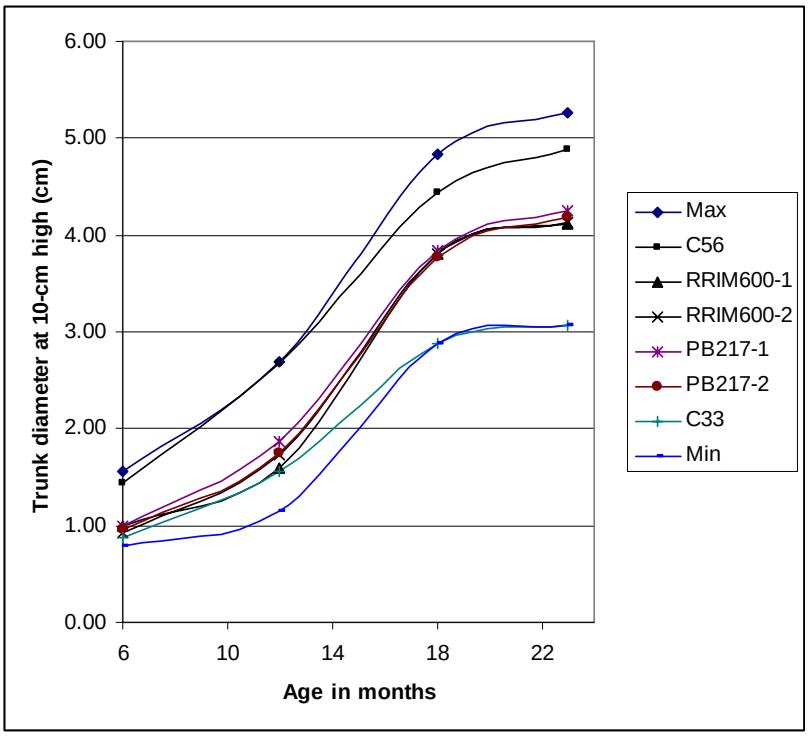


Figure 3 - Evolution of the trunk girth measured: A) at 1-meter high, and B) at 1.7-meter high from 18 to 47-month old (cm).

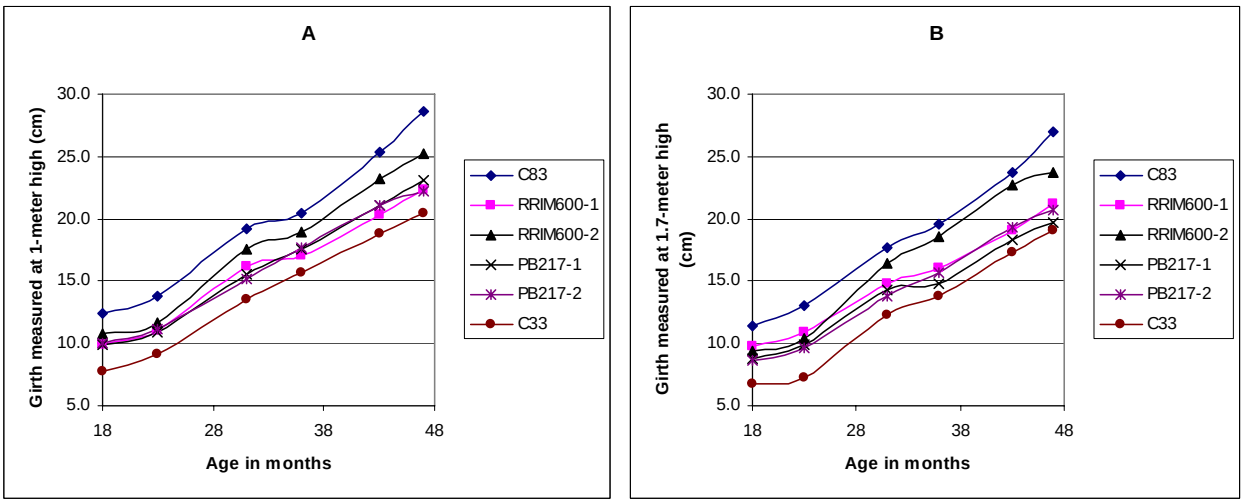


Figure 4 - Evolution of the growth in height over the first four years. A) Evolution of the height of the genotypes from 6 to 47-month old (cm). B) Relationship between the height at 17-month old and the height increment from 17 to 47-months old.

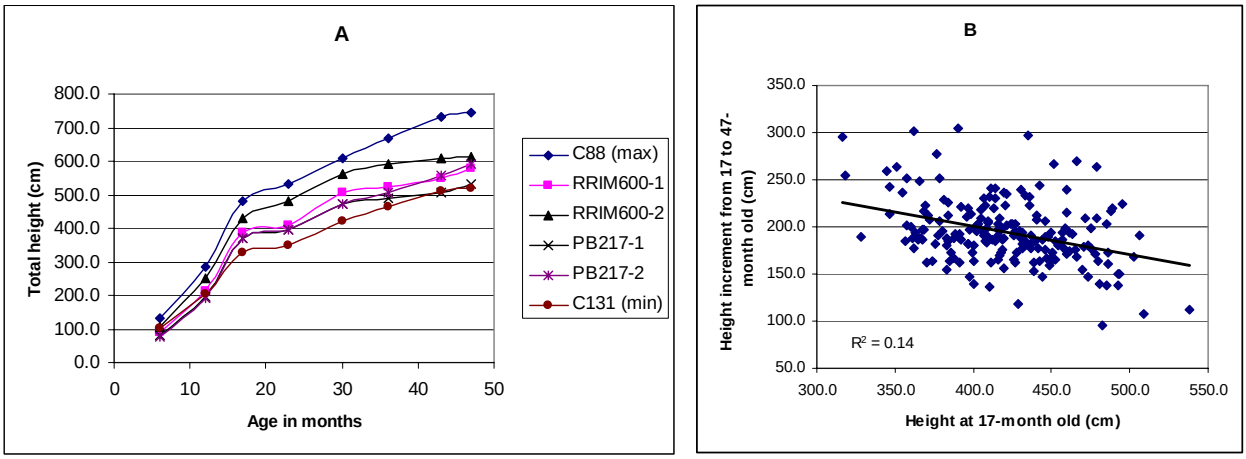


Figure 5 - A) : Relationship between the total height (at 36-month old) and the number of branches below 4-meter high at 42-month old (N4 counted over 1959 trees and distributed among 27 height-classes). B) : Relationship between total height and N4 among the 200 genotype-codes.

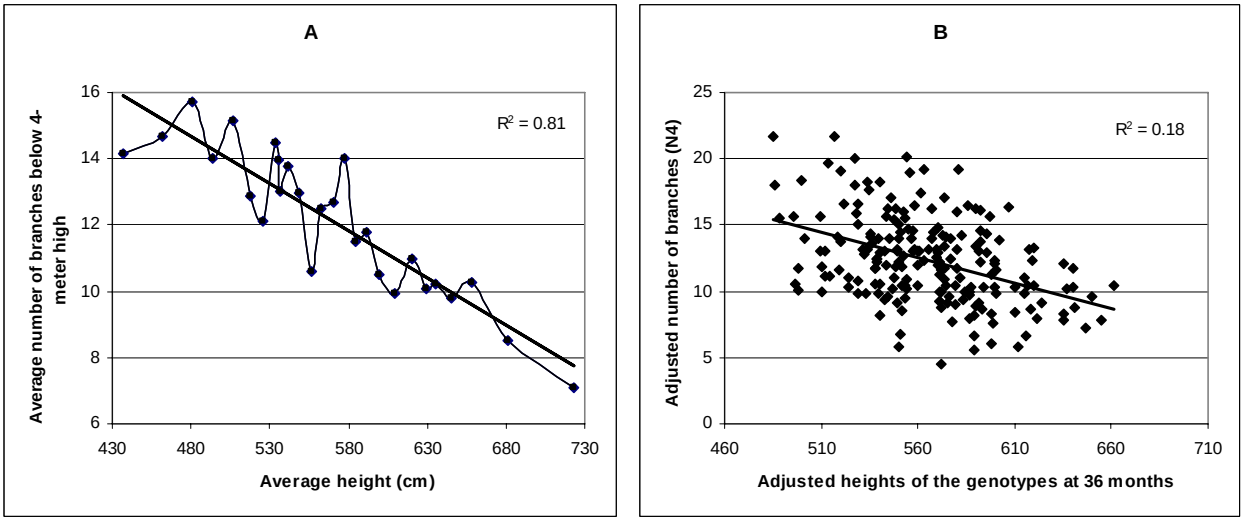


Figure 6 - Wintering earliness as observed at 31-month old during the dry season (January 2005). A) Variation of the index by decreasing rank over the 200 genotypes. B) Wintering earliness of some genotypes, including the parents RRIM600 and PB217.

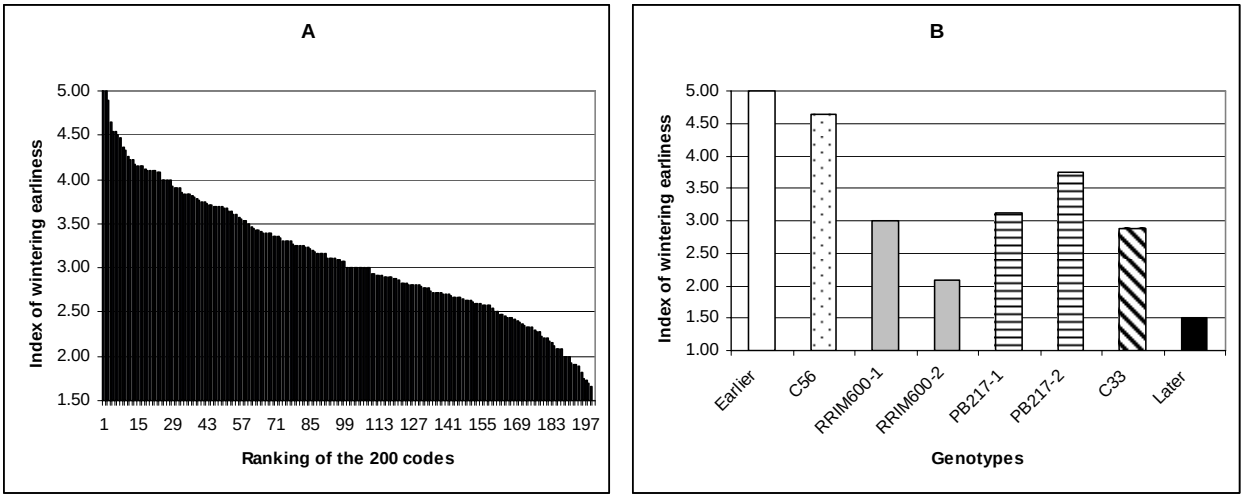


Figure 7 - Variation of fruiting ability at 3-year old. A) Variation of the index by decreasing rank over the 200 genotypes. B) Fruiting ability of some genotypes, including the parents RRIM600 and PB217.

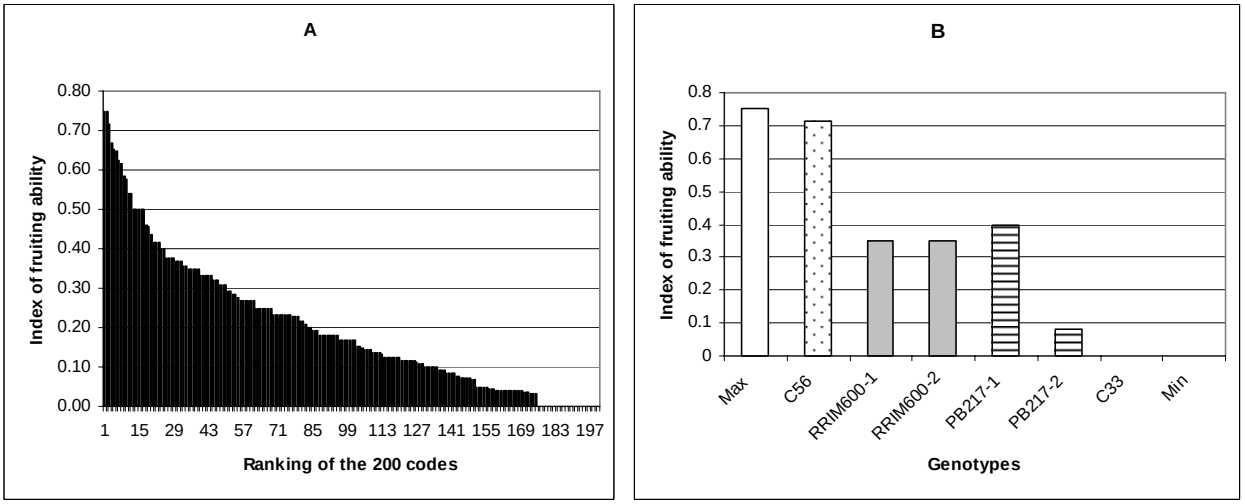
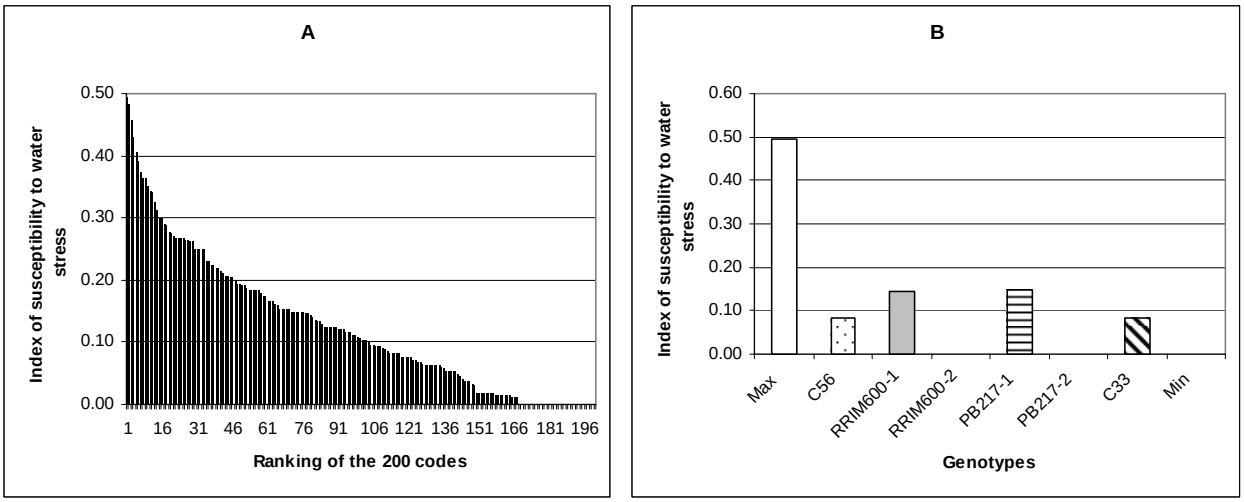


Figure 8 - Variation of the susceptibility to water stress as observed after the severe drought of 2004-2005. A) Variation of the index by decreasing rank over the 200 genotypes. B) Susceptibility to water stress of some genotypes, including the parents RRIM600 and PB217.



End.